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International application number: PCT/US04/043571

International filing date: 22 December 2004 (22.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/532,256
Filing date: 22 December 2003 (22.12.2003)

Date of receipt at the International Bureau: 11 February 2005 (11.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
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APPLICATION NUMBER: *60/532,256*
FILING DATE: *December 22, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/43571*

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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION Under 37 CFR 1.53 (b)(2).

Attorney Docket No.	570.P	Type a plus sign (+) inside this box ----->	+

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TITLE OF THE INVENTION (280 characters max)

Novel Analogs of Carbovir and Abacavir

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ENCLOSED APPLICATION PARTS (check all that apply)

[X] Specification — *Number of pages* 8
[ ] Small Entity Statement
[ ] Drawing(s) — *Number of sheets* _____
[ ] Other (specify)

METHOD OF PAYMENT (check one)

[X] The Commissioner is hereby authorized to charge filing fees (as well as any additional fees which may be required by this paper) and credit Deposit Account Number **07-1250**.

Provisional Filing Fee Amount ($) **$160.00**

The invention was made by an agency of the United States Government of under a contract with an agency of the United States Government.

[X] No.
[ ] Yes, the name of the U.S. Government Agency and the Government contract number are:

Respectfully submitted,

SIGNATURE _____ DATE December 22, 2003

TYPED or PRINTED NAME Alex Andrus REGISTRATION NO. (if appropriate) 44,509

[ ] Additional inventors are being named on separately numbered sheets attached hereto

PATENT

Attorney Docket No. 570.P

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Maria Fardis and Choung U. Kim

For: Novel Analogs of Carbovir and Abacavir

Mail Stop Provisional Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PROVISIONAL APPLICATION COVER SHEET
(37 C.F.R. § 1.51 (2) (i))

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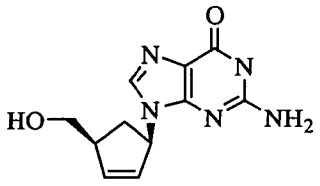
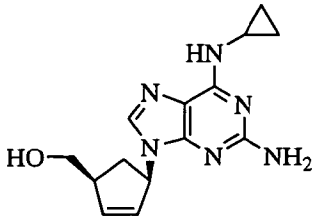
Novel Analogs of Carbovir and Abacavir

Carbovir (Structure below, EP 00,434,450 and EP 00,349,242) along with abacavir are well known anti-HIV carbocyclic nucleosides. GlaxoSmithKline has developed and launched the cytosine nucleoside analog and nucleoside reverse transcriptase inhibitor (NRTI) abacavir (ziagen) for the treatment of HIV infection. Abacavir is the most potent NRTI developed to date. An average reduction in viral load of more than 1.4 log10 RNA copies/ml is observed after a short course of abacavir monotherapy.



Carbovir



Abacavir

In vitro, resistant virus is not rapidly selected by abacavir. A significant decrease in susceptibility to abacavir in wild-type or zidovudine-resistant HIV-1 strains was not observed until after eight to ten passages in MT-4 cells. A set of resistance mutations at HIV reverse transcriptase (RT) codons, 65R, 74V, 115F and/or 184V, are selected during

in vitro passage with abacavir, and a combination of these mutations was required to confer a 10-fold reduction in abacavir susceptibility in a laboratory strain of HIV. The first mutation detected upon passage of HIV-1 in an increasing concentration of abacavir is M184V, which confers only a 3-fold decrease in HIV-1 susceptibility. Phenotype resistance to 3TC and/or the presence of the 184V mutation does not prevent viral load response to abacavir therapy. Resistance to multiple nucleosides is associated with a decreased or absent response to abacavir.

The anabolism of abacavir was characterized in human T-lymphoblastoid CD4+ CEM cells. Abacavir was ultimately anabolized to the triphosphate (TP) of carbovir. However, less than 2% of intracellular abacavir was converted to carbovir, an amount insufficient to account for the levels of TP observed. Abacavir is anabolized to its 5'-monophosphate (MP) by the recently characterized enzyme, adenosine phosphotransferase, but neither its diphosphate (DP) nor its TP was detected. The MP, DP, and TP of carbovir were found in cells incubated with either abacavir or carbovir, with carbovir-TP the major phosphorylated species. Carbovir is phosphorylated by 5'-nucleotidase and mycophenolic acid increased the formation of carbovir-TP from carbovir by 75-fold. Abacavir-MP was not a substrate for adenylate deaminase but was a substrate for a distinct cytosolic deaminase that was inhibited by 2'-deoxycoformycin-5'-MP. Thus, abacavir is phosphorylated by adenosine phosphotransferase to abacavir-MP, which is converted by a novel cytosolic enzyme to carbovir-MP. Carbovir-MP is then further phosphorylated to carbovir-TP by cellular kinases. The 5'-TP of carbovir was a potent, selective inhibitor of HIV RT, with Ki values for DNA polymerases α, β, γ and ε which were 90-, 2900-, 1200-, and 1900-fold greater, respectively, than for RT (Ki = 21 nM).

Abacavir was well-absorbed, with a relative bioavailability of 96% with the caplet formulation. In humans, a 5'-carboxylic acid and a 5'-glucoronide are the major metabolites of abacavir measured in plasma and urine. On average, less than 3% of the dose is excreted in urine unchanged, or as hydroxylated metabolites. The major pathways for the metabolism of abacavir indicate potential for metabolic interaction

when it is co-administered with drugs metabolized by ADH or UDPGT. Even though P450 oxidation appears to be a minor pathway of abacavir metabolism, it is a major metabolic pathway for many HIV therapies, acting predominantly through the microsomal cytochrome P450 isozyme, 3A4. In vitro studies in human liver slices were predictive of the effects of ethanol on the metabolism of abacavir in humans, including inhibition of formation of the carboxylic acid metabolite and a compensatory increase in formation of the glucuronide metabolite; these studies predicted no effect of amprenavir on abacavir metabolism in human.

Abacavir was well-tolerated by all subjects, with mild to moderate asthenia, abdominal pain, headache, diarrhea, and dyspepsia being the most frequently reported adverse events. Abacavir can be taken on a twice-daily dosing schedule as a 300 mg tablet and 20 mg/ml liquid formulation, with no dietary restrictions and no known drug-drug interactions.

Dideoxynucleotide use such as dideoxycytidine (ddC) and of didehydrodideoxythymidine (d4T) is limited by associated painful sensory-motor peripheral neuropathy. Dideoxyinosine also shares this complication as well as causing acute pancreatitis, and hepatotoxicity in some cases (Maag, H. et al., *J. Med. Chem.*, **1992**, *35*, 1440). Yet another concern about this class of compounds has been the emergence of resistant HIV strains in patients undergoing treatment with nucleosides. For instance the ddI-resistant strains were also shown to be resistant to ddC. In another study, clinical HIV isolates resistant to AZT displayed marked resistance to d4T. It appears, then, that some cross resistance is inevitable among this class of similar nucleoside structures. Therefore, an important criterion for the design of any new nucleoside drug would be a distinct dissimilarity of structure to the current family of dideoxynucleosides.

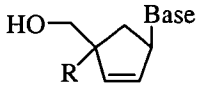
Substitution at the 4' position of nucleosides have been reported to exert potent antiviral activity against HIV-1 as demonstrated by 4'-azido-2'-deoxythymidine (4'-AZT) (Maag, H. et al., *J. Med. Chem.*, **1992**, *35*, 1440). In addition, 4'-ethynyl nucleoside analogs were shown to be active against a wide spectrum of HIV viruses, specifically against

HIV-1 $_{M184V}$ and HIV-1 $_{M184I}$ (Kodama, E. I. et al., *Antimicrob. Agents Chemother.*, **2001**, 1539). Recently, Haraguchi et al. reported the preparation of 4'-substituted d4T analogs. They demonstrated that contrary to the previous reports, the 3'-hydroxy group is not necessary for the 4'-substituted nucleosides to be active against HIV (Haraguchi, K. et al., *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 3775). Therefore, we believe that such substitutions on a carbovir cyclopentene ring also provide potent anti-HIV compounds. A series of 4' substituted carbocyclic nucleosides carrying a variety of bases at the 1' position are described herein. A more specific example depicting the general formula described in figure 1 is also shown. The 4' substitution may include alkyl, alkenyl, alkynyl, azido, and cyano groups.

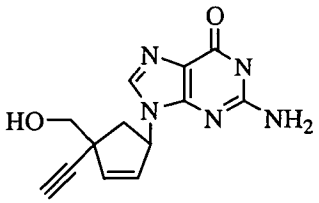
Figure 1:

Base= refers to the commonly known nucleoside bases.

R= -C≡CH, CH_2=CH_2, CN, N_3, Me, CH_2F.



General structure of nucleosides claimed in this document.

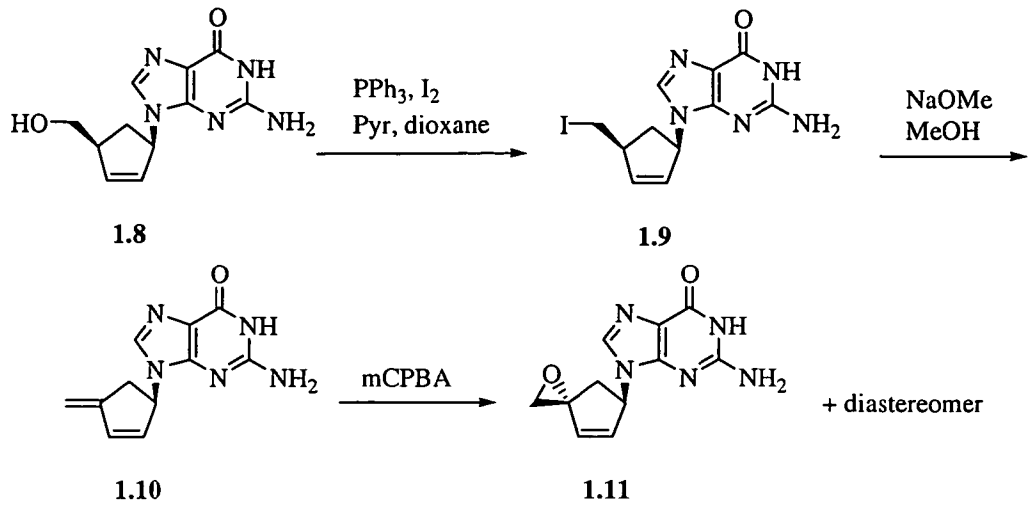


1

A specific nucleoside in which base is G.

Compounds such as 1 can be prepared according to Schemes 1 and 2.

Scheme 1:



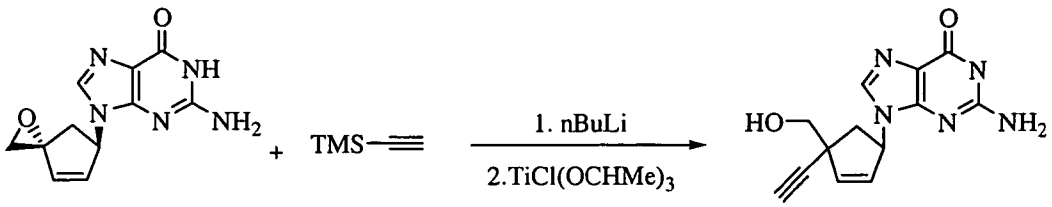
1.8 **1.9**

1.10 **1.11**

Preparation of compound **1** follows literature reactions that lead to carbovir **1.8** (Crimmins, M. T. et al., *J. Org. Chem.*, **1996**, *61*, 4192). Selective iodination at 5' proceeds to provide compound **1.9** using triphenylphosphine, iodine, and either pyridine or imidazole in dioxane (Maag, H. et al., *J. Med. Chem.*, **1992**, *35*, 1440). Dehydrohalogenation of 5'-halonucleosides has been well-described (Ueda, T. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press; New York, 1988; 83). Use of sodium methoxide proceeds to generate the eliminated product **1.10**. Epoxidation of the exocyclic olefin proceeds without a stereoselection to provide a mixture of two diastereomers. Such reactions may be performed using *m*-chloroperbenzoic acid or Corey's reagent (dimethylsulfoxonium methylide) (Gololobov, Y. G. et al., *Tetrahedron*, **1997**, *43*, 12, 2609).

The mixture of diastereomers can be opened using ethynyl-trimethyl-silane in the presence of titanium acetylide at the more highly substituted carbon atom exclusively as reported by Krause, N. et al., *Chem. Ber.*, **1988**, *121*, 7, 1315 to provide the desired product **1** (Scheme 2).

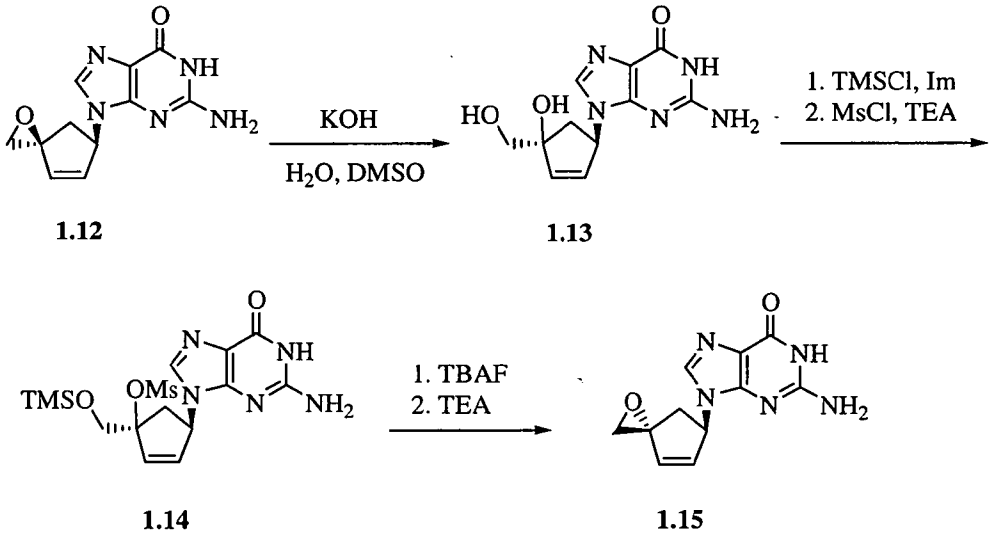
Scheme 2:



1.11 **1**

The undesired diastereomer of the epoxidation reaction, compound **1.12**, can readily be converted to the desired diastereomer using a four step sequence (Scheme 3). The undesired epoxide is opened using basic condition such as sodium hydroxide in water and DMSO (Lepage, O. et al., *J. Org. Chem.*, **2003**, *68*, 6, 2183) to provide a diol. The primary alcohol can then be protected with a silyl group and the 3' alcohol can be turned into a leaving group such as a mesylate. Release of the silyl group followed by exposure of the primary alcohol to base will lead to ring closure to form the desired diastereomer of the epoxide. Conversion of the desired epoxide to the product **1** is shown in Scheme 2.

Scheme 3:

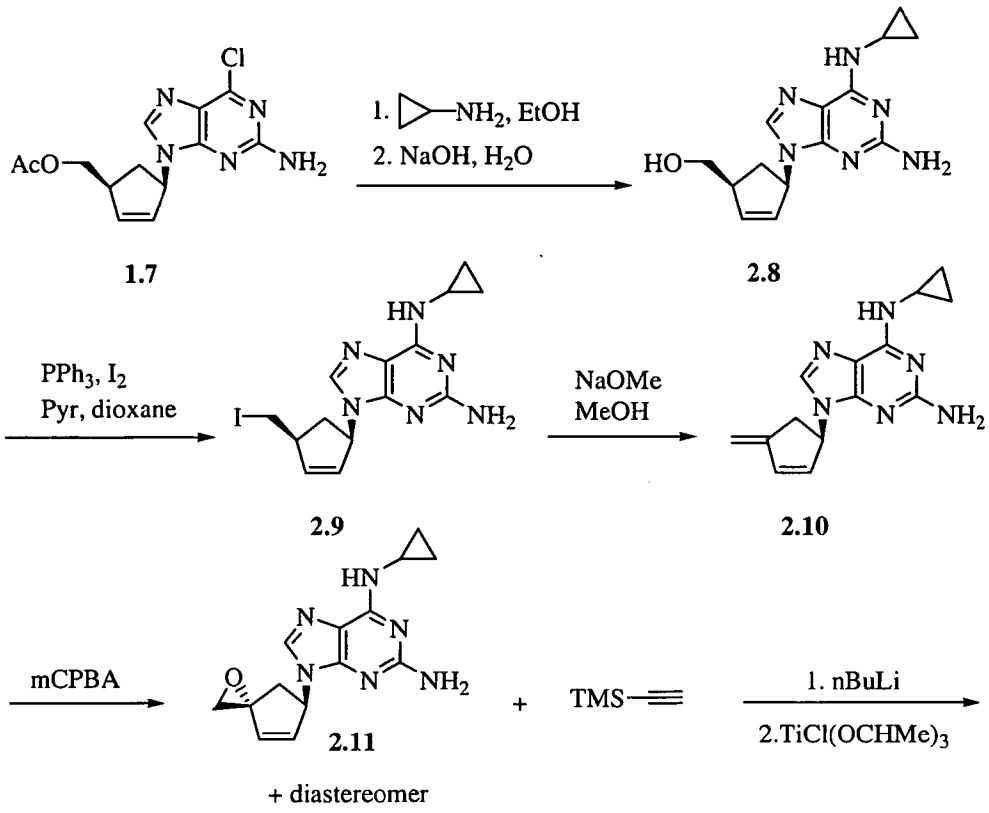


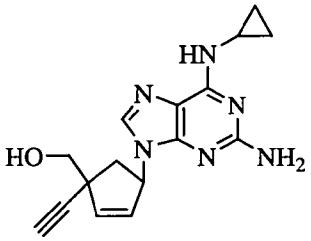
1.12 **1.13**

1.14 **1.15**

This methodology can be applied to provide the 4'-alkynyl abacavir, compound **2**, as demonstrated in Scheme 4:

Scheme 4:





2

Formation of Abacavir **2.8** can be achieved from a common intermediate **1.7** according to Crimmins, M. T. et al., *J. Org. Chem.*, **1996**, *61*, 4192. Selective iodination at 5' proceeds to provide compound **2.9** using triphenylphosphine, iodine, and either pyridine or imidazole in dioxane (Maag, H. et al., *J. Med. Chem.*, **1992**, *35*, 1440). Dehydrohalogenation of 5'-halonucleosides has been well-described (Ueda, T. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press; New York, 1988; 83). Use of sodium methoxide proceeds to generate the eliminated product **2.10**. Epoxidation of the exocyclic olefin proceeds without a stereoselection to provide a mixture of two diastereomers. Such reactions may be performed using *m*-

chloroperbenzoic acid or Corey's reagent (dimethylsulfoxonium methylide) (Gololobov, Y. G. et al., *Tetrahedron*, **1997**, *43*, 12, 2609). The mixture of diastereomers can be opened using ethynyl-trimethyl-silane in the presence of titanium acetylide at the more highly substituted carbon atom exclusively as reported by Krause, N. et al., *Chem. Ber.*, **1988**, *121*, 7, 1315 to provide the desired product **2** (Scheme 4).

The same reaction scheme can be used to prepare 4' cyano and azido analogs of carbovir or abacavir. In order to prepare the 4' ethylene analog, a reduction of the acetylene to ethylene can be performed (for an example see Shinjiro, S., et al., *Org. Lett.*, **2003**, *5*, 11, 1891).